Penicillin-binding protein folding is dependent on the PrsA peptidyl-prolyl cis-trans isomerase in Bacillus subtilis

Hyyrylainen, Hanne-Leena; Marciniak, Bogumila C.; Dahncke, Kathleen; Pietiainen, Milla; Courtin, Pascal; Vitikainen, Marika; Seppala, Raili; Otto, Andreas; Becher, Doerte; Chapot-Chartier, Marie-Pierre

Published in:
Molecular Microbiology

DOI:
10.1111/j.1365-2958.2010.07188.x

Publication date:
2010

GFP-MreC is independent of PrsA

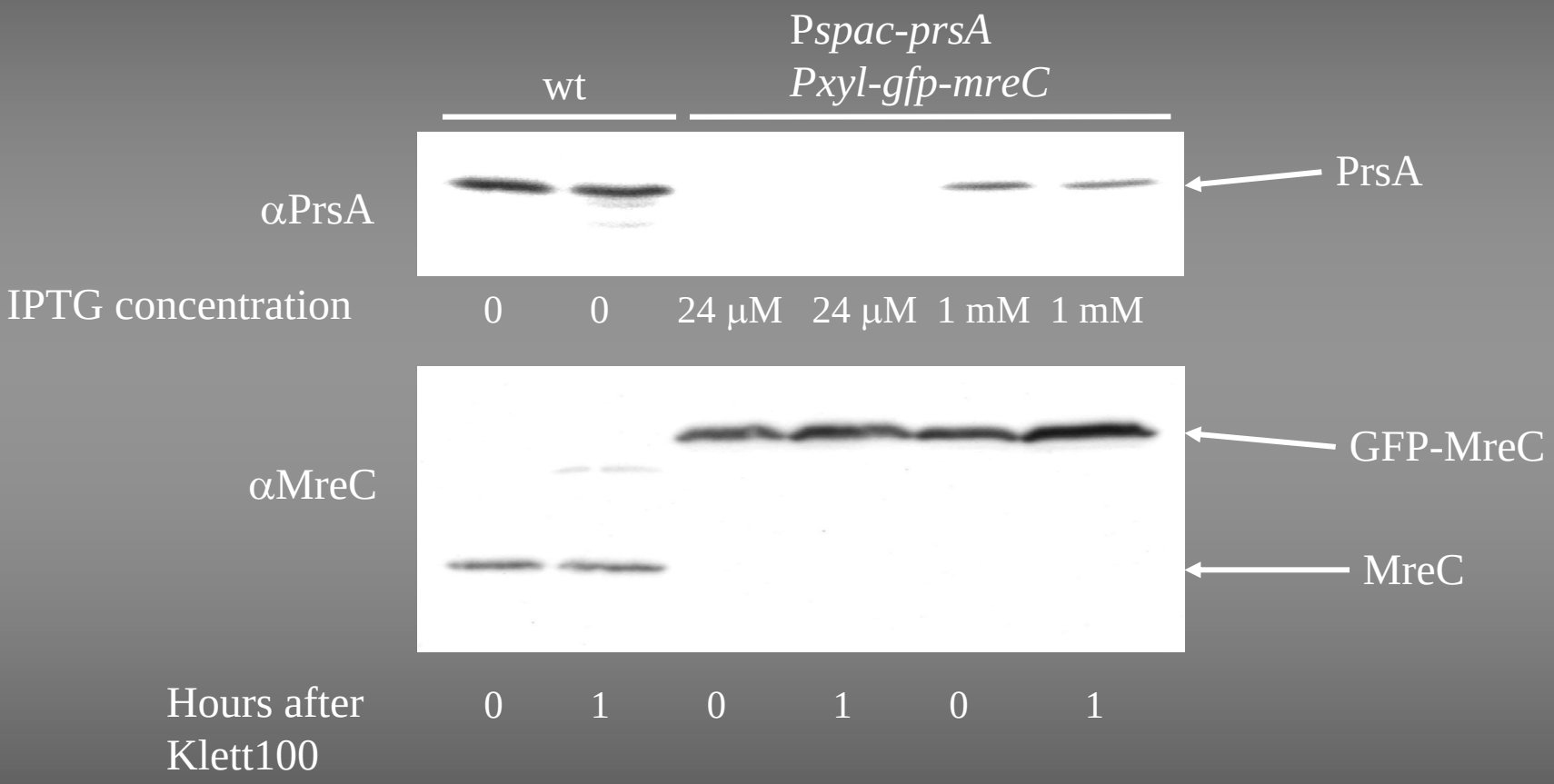


Fig.S1

MreC is independent of PrsA

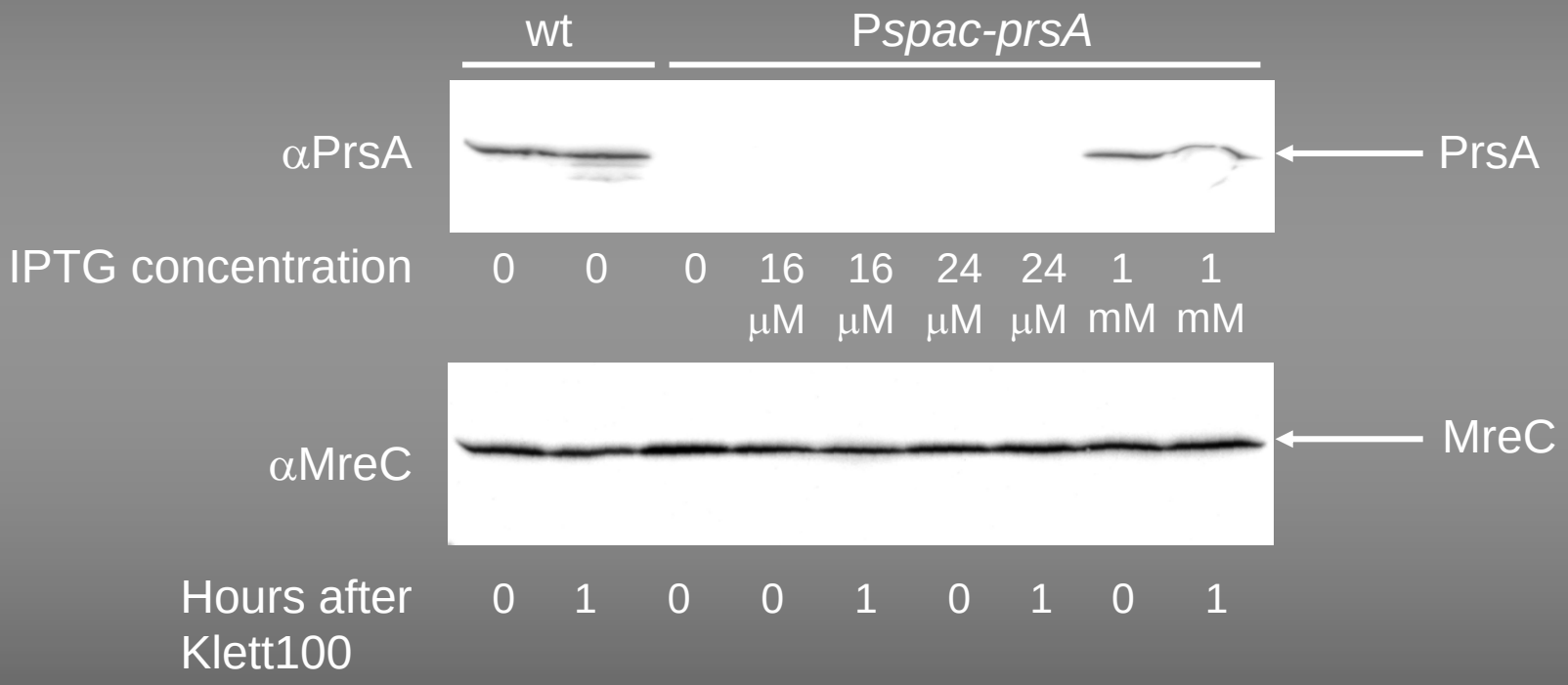


Fig.S1

GFP-PBP2a levels in cell membranes

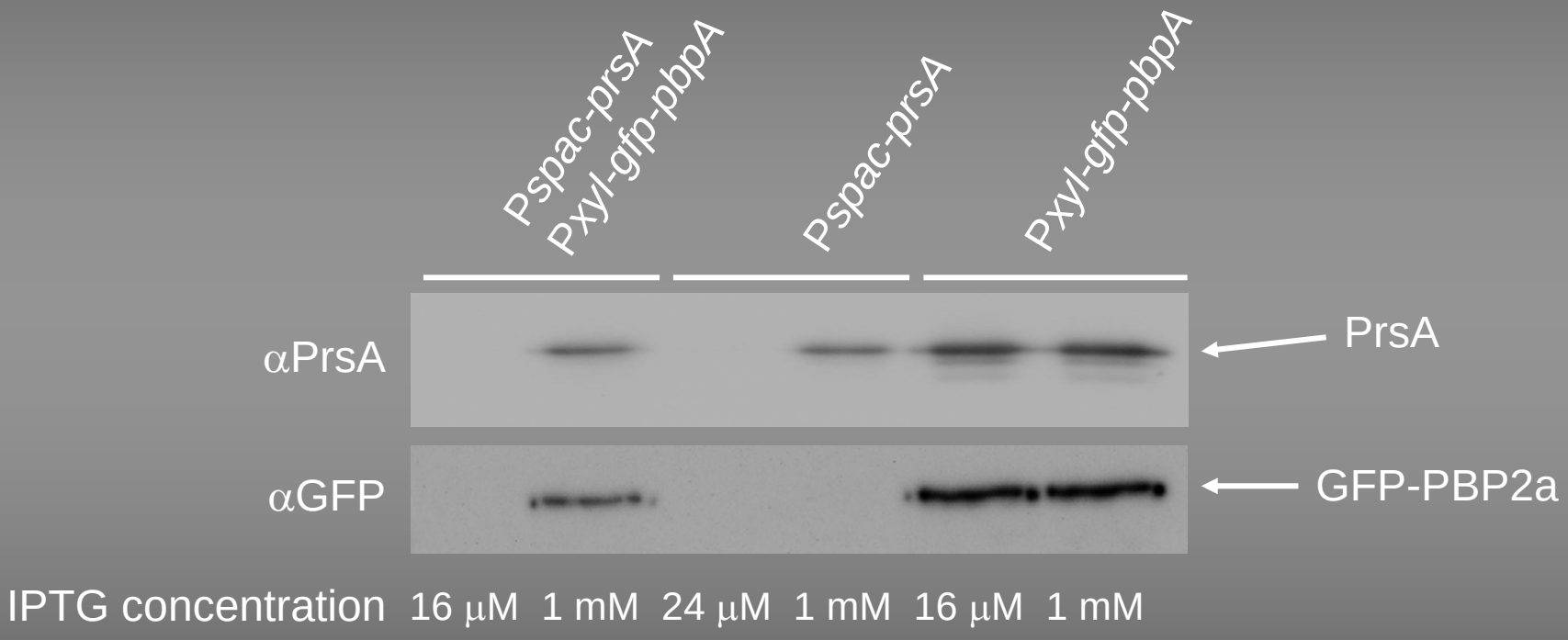


Fig. S1

GFP-PBP1 is independent of PrsA

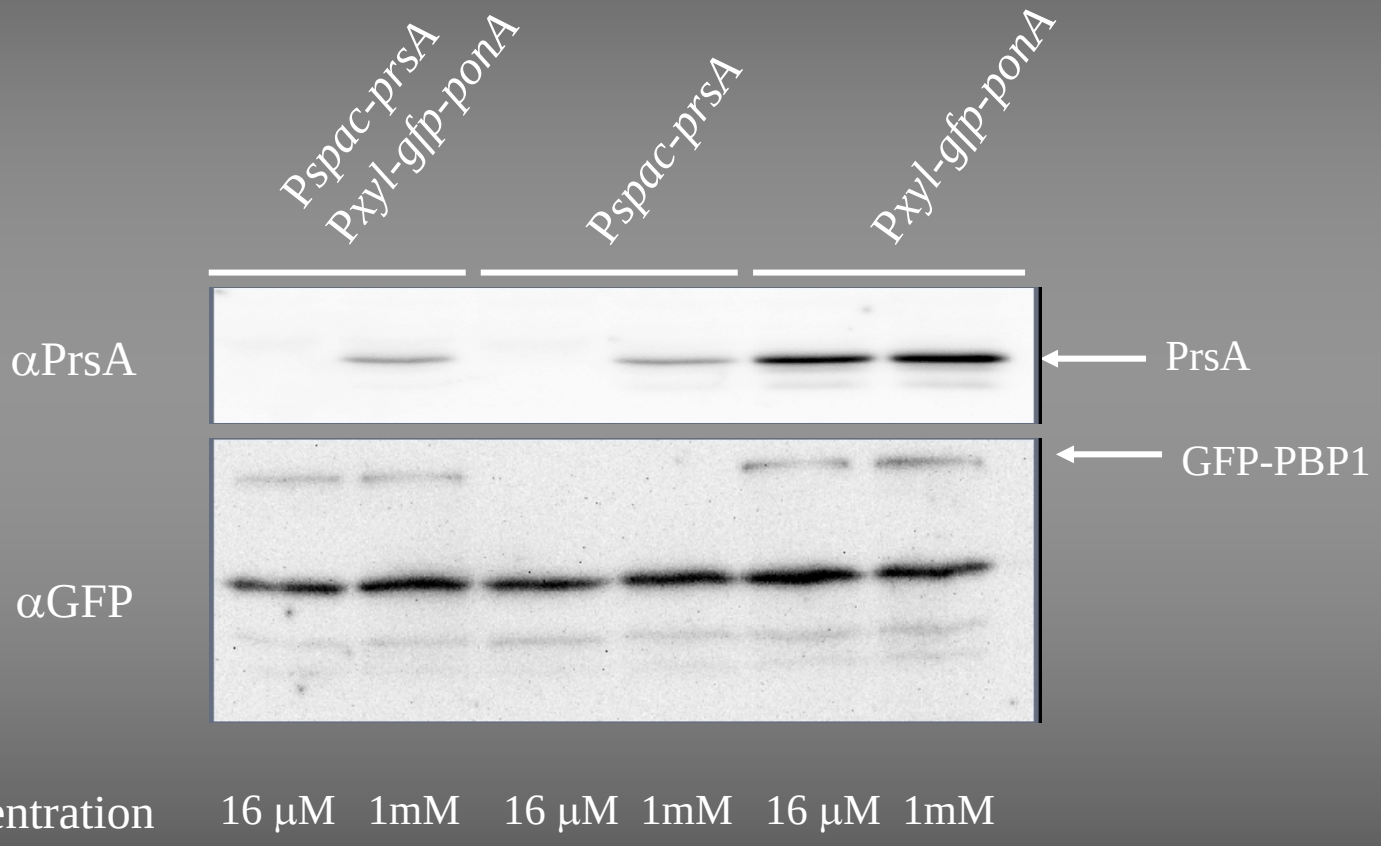
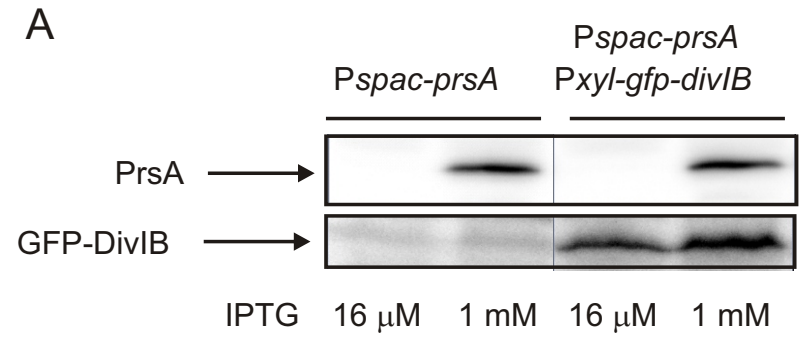
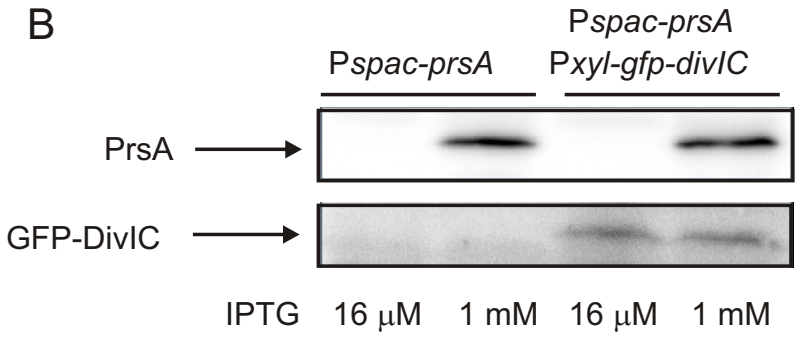


Fig. S1

Fig. S2


A
Pspac-prsA
Pspac-prsA
Pxyl-gfp-divIB
PrsA
GFP-DivIB
IPTG
16 μM
1 mM
16 μM
1 mM


B
Pspac-prsA
Pspac-prsA
Pxyl-gfp-divIC
PrsA
GFP-DivIC
IPTG
16 μM
1 mM
16 μM
1 mM

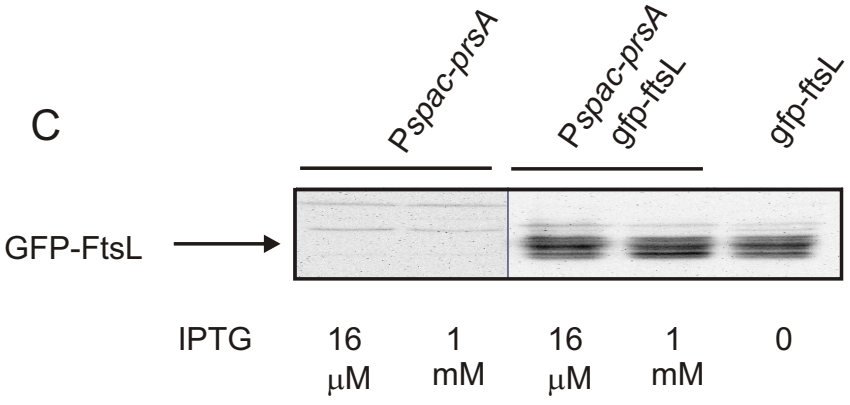

C
Pspac-prsA
Pspac-prsA
gfp-ftsL
gfp-ftsL
GFP-FtsL
IPTG
16 μM
1 mM
16 μM
1 mM
0

Fig. S3

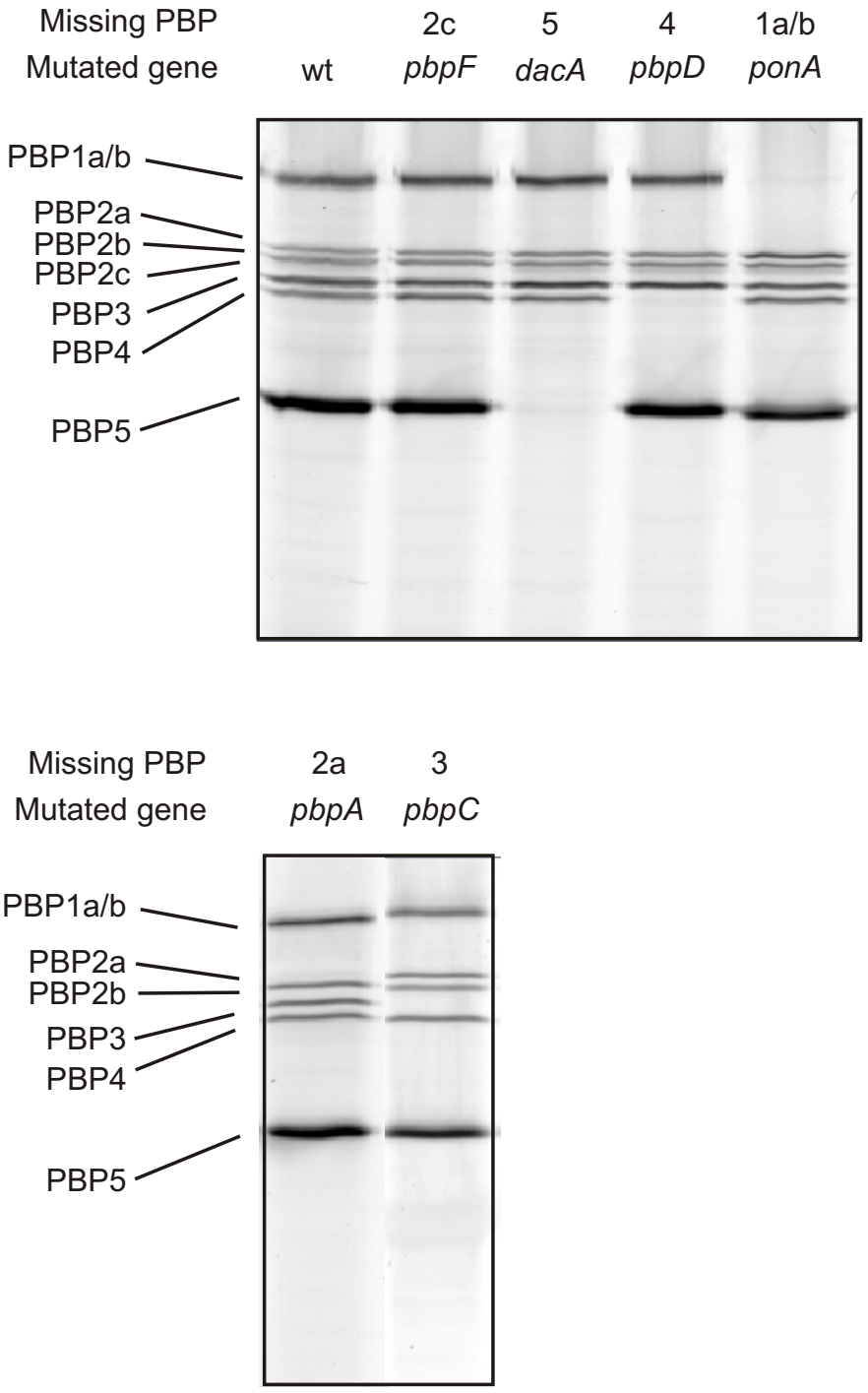
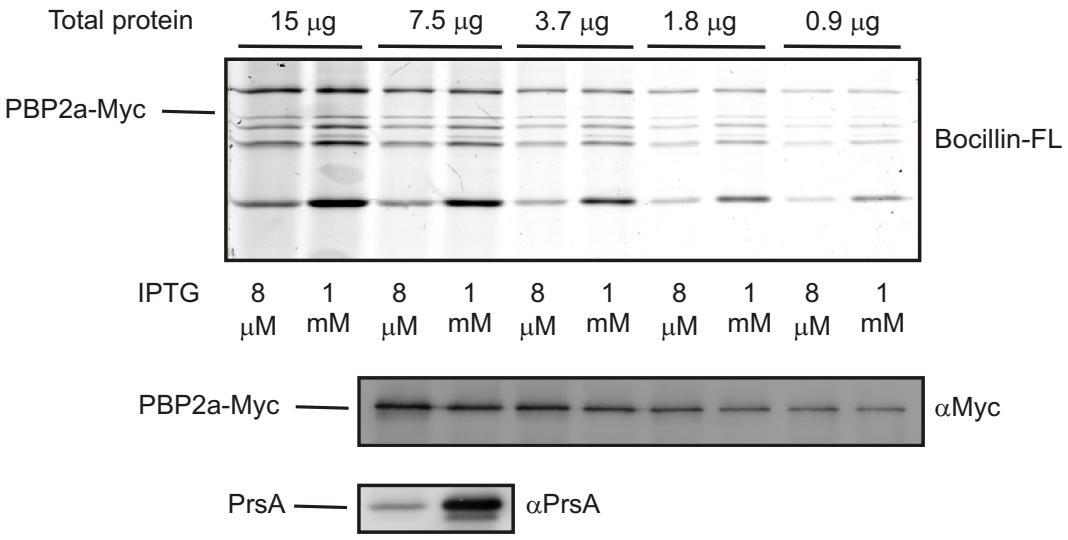


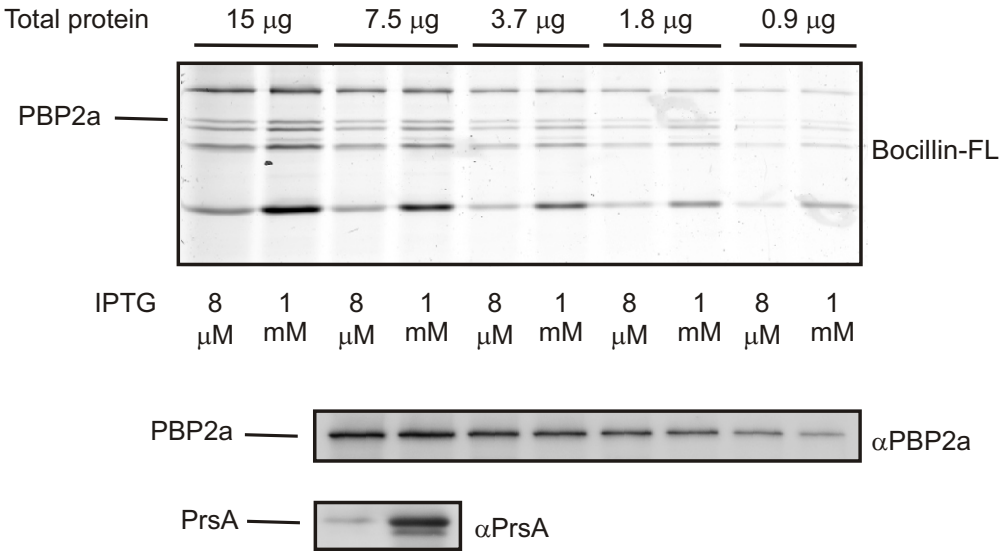
Fig. S3. Identification of the main PBPs by null mutations of the PBP genes and Bocillin-FL staining (see also (Popham and Setlow, 1996)). Cytoplasmic membranes were isolated and PBPs were visualized by staining with Bocillin-FL (see Experimental procedures) and separation in SDS-PAGE. The identification of PBP2b is based on its known migration next to PBP2a (Popham and Setlow, 1996).

Popham, D. L., and Setlow, P. (1996) Phenotypes of *Bacillus subtilis* mutants lacking multiple class a high-molecular-weight penicillin-binding proteins. *J Bacteriol* **178**: 2079-2085.

A


Total protein
15 μg
7.5 μg
3.7 μg
1.8 μg
0.9 μg
PBP2a-Myc
Bocillin-FL
IPTG
8 μM
1 mM
PBP2a-Myc
αMyc
PrsA
αPrsA

B


Total protein
15 μg
7.5 μg
3.7 μg
1.8 μg
0.9 μg
PBP2a
Bocillin-FL
IPTG
8 μM
1 mM
PBP2a
αPBP2a
PrsA
αPrsA

C

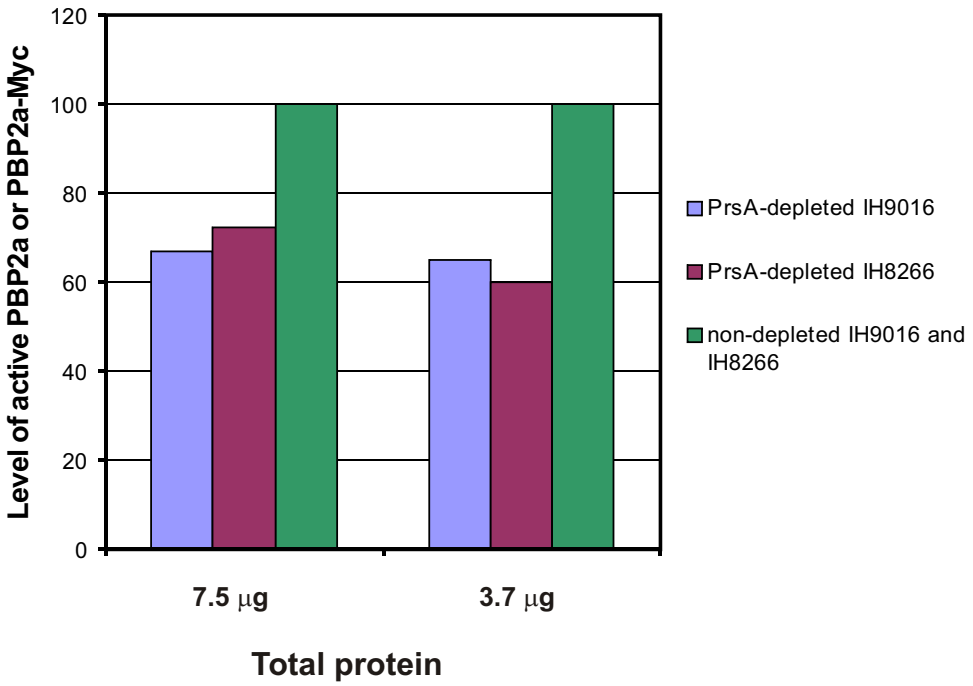


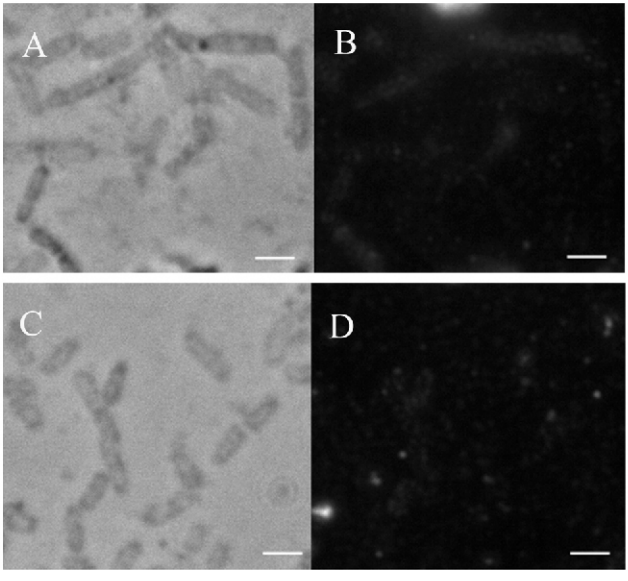
Fig. S4. Quantitative analysis of PBP2a misfolding in PrsA-depleted cells. *cssS* mutant strains were used to prevent proteolytic degradation of the misfolded PBP2a, so that it could be identified and quantitated by Bocillin-FL staining (active PBP2a) and immunoblotting (total PBP2a: active and inactive).

A. Membrane samples were isolated from PrsA-depleted and non-depleted cells of *B. subtilis* IH9016 (*pbpA-myc* P*spac-prsA cssS*::*spec*) and a series of two-fold diluted samples were subjected to SDS-PAGE and immunoblotted with anti-c-Myc antibodies. The amounts of full-length PBP2a-Myc were quantitated by determining optical densities of the PBP2a-Myc bands in lanes loaded with 7.5 µg and 3.7 µg of total membrane protein. The same dilution series of the membranes were also stained with Bocillin-FL and fluorescence intensities in full-length PBP2a-Myc were determined to quantitate correctly-folded active PBP2a-Myc.

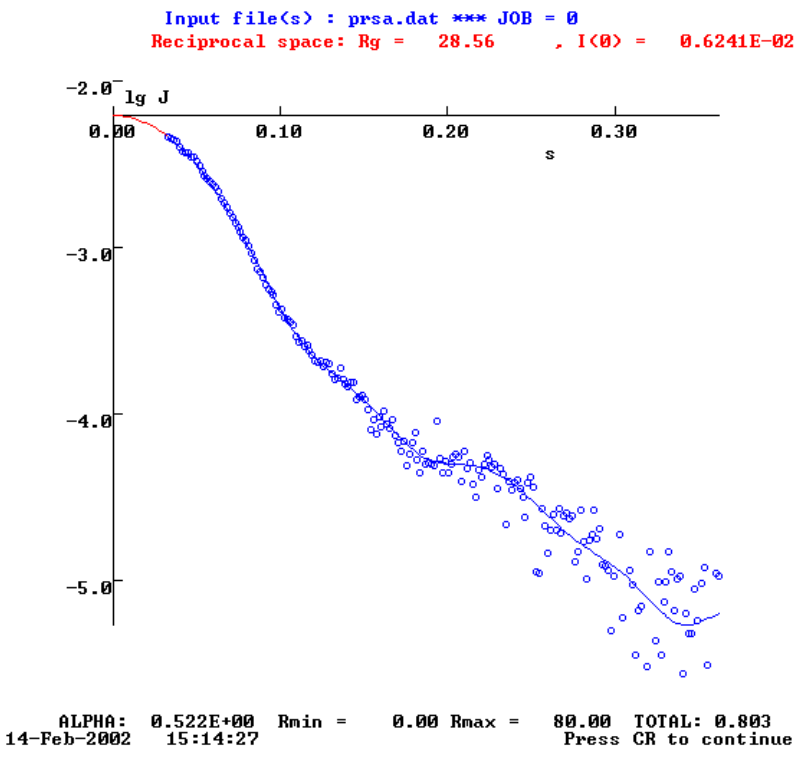
B. The same analysis was also performed with *B. subtilis* IH8266 (P*spac-prsA cssS*::*spec*) and anti-PBP2a antibodies.

C. Ratios of correctly-folded active PBP2a-Myc or PBP2a as determined by Bocillin-FL staining to total PBP2a-Myc or PBP2a as determined by immunoblotting were calculated, respectively. The ratios (level of active PBP2a-Myc or PBP2a) of non-depleted cells were adjusted to 100. The proportion of misfolded PBP2a-Myc or PBP2a in PrsA-depleted cells as compared to non-depleted cells was 30-40%. Fig. 3E suggests that the level of active PBP2a-Myc is lower in the PrsA-depleted *cssS* wild-type strain than in the PrsA-depleted *cssS* mutant, most probably implying that if misfolded PBP2a is not degraded due to the absence of the quality control proteases, at least a portion of this can slowly fold to an active conformation in the absence of the folding assistance of PrsA.

Fig. S5



Small-angle X-ray scattering (SAXS) characterization of PrsA



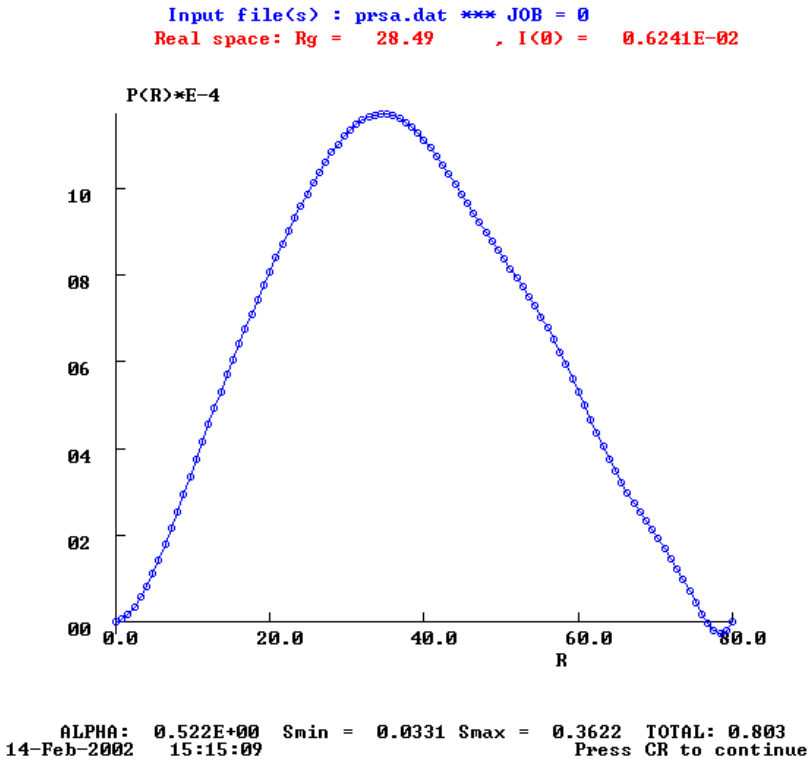


Fig.S6. The radius of gyration (*Rg*) is 27-29 Å, which is considerably larger than expected for a monomer. The measured *Rg* corresponds more like a tetramer.

Table S2. Plasmids used in this study.

Plasmid	Characteristics	Reference
pKTH3384	Derivative of pMUTIN4; P*spac*-'*prsA´*; Em^r	(Vitikainen *et al.*, 2001)
pKTH3805	Derivative of pMUTIN-cMyc; *´prsA-myc*; Em^r	This study
pKTH3806	Derivative of pSG4902; P*xyl-gfp-´divIC´*; Cm^r	This study
pKTH3814	Derivative of pSG4902; P*xyl-gfp-´divIB´*; Cm^r	This study
pKTH3828	Derivative of pSG4902; *´pbpA-myc*; Cm^r	This study
pKTH3831	Derivative of pSG4902; *´dacA-myc*; Cm^r	This study
pMUTIN-cMyc	Integration vector; Em^r	(Kaltwasser *et al.*, 2002)
pSG4902	Integration vector; Cm^r	(Scheffers *et al.*, 2004)

Kaltwasser, M., Wiegert, T., and Schumann, W. (2002) Construction and application of epitope- and green fluorescent protein-tagging integration vectors for *Bacillus subtilis*. *Appl Environ Microbiol* **68**: 2624-2628.

Scheffers, D. J., Jones, L. J., and Errington, J. (2004) Several distinct localization patterns for penicillin-binding proteins in *Bacillus subtilis*. *Mol Microbiol* **51**: 749-764.

Vitikainen, M., Pummi, T., Airaksinen, U., Wu, H., Sarvas, M., and Kontinen, V. P. (2001) Quantitation of the capacity of the secretion apparatus and requirement for PrsA in growth and secretion of α-amylase in *Bacillus subtilis*. *J Bacteriol* **183**: 1881-1890.